Amendments to the Claims

1. (original) A 2μm-family plasmid comprising a polynucleotide sequence insertion, deletion and/or substitution between the first base after the last functional codon of at least one of either a *REP2* gene or an *FLP* gene and the last base before the FRT site in an inverted repeat adjacent to said gene.

2. (original) The 2μm-family plasmid of Claim 1 wherein, other than the polynucleotide sequence insertion, deletion and/or substitution, the *FLP* gene and/or the *REP2* gene has the sequence of a *FLP* gene and/or a *REP2* gene, respectively, derived from a naturally occurring 2μm-family plasmid.

3. (original) The 2μm-family plasmid of Claim 1 wherein the naturally occurring 2μm-family plasmid is selected from pSR1, pSB3 or pSB4 as obtained from *Zygosaccharomyces rouxii*, pSB1 or pSB2 both as obtained from *Zygosaccharomyces bailli*, pSM1 as obtained from *Zygosaccharomyces fermentati*, pKD1 as obtained from *Kluyveromyces drosophilarum*, pPM1 as obtained from *Pichia membranaefaciens*, and the 2μm plasmid as obtained from *Saccharomyces cerevisiae.*

4. (currently amended) The 2μm-family plasmid of Claim 2 ~~or 3~~ wherein the sequence of the inverted repeat adjacent to said *FLP* and/or *REP2* gene is derived from the sequence of the corresponding inverted repeat

in the same naturally occurring 2μm-family plasmid as the sequence from which the gene is derived.

5. (currently amended) The 2μm-family plasmid of ~~any one of~~ Claims 2 ~~to 4~~ wherein the naturally occurring 2μm-family plasmid is the 2μm plasmid as obtained from *Saccharomyces cerevisiae*.

6. (original) The 2μm-family plasmid of Claim 5 wherein the polynucleotide sequence insertion, deletion and/or substitution occurs at a position between the first base of codon 59 of the *REP2* gene and the last base before the FRT site in the adjacent inverted repeat.

7. (currently amended) The 2μm-family plasmid of Claim 5 ~~or 6~~ wherein, other than the polynucleotide sequence insertion, deletion and/or substitution, the sequence of the *REP2* gene and the adjacent inverted repeat is as defined by SEQ ID NO:1 or variant thereof.

8. (currently amended) The 2μm-family plasmid of ~~any one of~~ Claims 1 ~~to 7~~ wherein polynucleotide sequence insertion, deletion and/or substitution occurs at a position between the first base of the inverted repeat and the last base before the FRT site.

9. (currently amended) The 2μm-family plasmid of ~~any one of~~ Claims 1 ~~to 7~~ wherein the polynucleotide sequence insertion, deletion and/or substitution occurs between the first base after the end of the *REP2*

coding sequence and the last base before the FRT site, such as at the first base after the end of the *REP2* coding sequence.

10. (currently amended) The 2μm-family plasmid of ~~any one of~~ Claims 1 ~~to 7~~ wherein, other than the polynucleotide sequence insertion, deletion and/or substitution, the inverted repeat that follows the *REP2* coding sequence has a sequence derived from the corresponding region of the 2μm plasmid as obtained from *Saccharomyces cerevisiae* and preferably the polynucleotide sequence insertion, deletion and/or substitution occurs at an *XcmI* site or an *FspI* site within the inverted repeat.

11. (original) The 2μm-family plasmid of Claim 5 wherein the polynucleotide sequence insertion, deletion and/or substitution occurs at a position between the first base of codon 344 of the *FLP* gene and the last base before the FRT site in the adjacent inverted repeat.

12. (currently amended) The 2μm-family plasmid of Claim 5 ~~or 11~~ wherein, other than the polynucleotide sequence insertion, deletion and/or substitution, the sequence of the *FLP* coding sequence and the adjacent inverted repeat is as defined by SEQ ID NO:2 or variant thereof.

13. (currently amended) The 2μm-family plasmid of Claim 11 ~~or 12~~ wherein the polynucleotide sequence insertion, deletion and/or

substitution occurs at a position between the first base of the inverted repeat and the last base before the FRT site.

14. (original) The 2μm-family plasmid of Claim 13 wherein the polynucleotide sequence insertion, deletion and/or substitution occurs at a position between the first base after the end of the *FLP* coding sequence and the last base before the FRT site.

15. (original) The 2μm-family plasmid of Claim 14 wherein the polynucleotide sequence insertion, deletion and/or substitution occurs at the first base after the end of the *FLP* coding sequence.

16. (currently amended) The 2μm-family plasmid of ~~any one of~~ Claims 11 ~~to 15~~ wherein, other than the polynucleotide sequence insertion, deletion and/or substitution, the inverted repeat that follows the *FLP* gene has a sequence derived from the corresponding region of the 2μm plasmid as obtained from *Saccharomyces cerevisiae*, and preferably the polynucleotide sequence insertion, deletion and/or substitution occurs at an *HgaI* site or an *FspI* site within the inverted repeat.

17. (currently amended) The 2μm-family plasmid of ~~any one of the preceding claims~~ Claim 1 comprising polynucleotide sequence insertions, deletions and/or substitutions between the first bases after the last functional codons of both of the *REP2* gene and the *FLP* gene and the last bases before the FRT sites in the inverted repeats adjacent

to each of said genes, which polynucleotide sequence insertions, deletions and/or substitutions can be the same or different.

18. (currently amended) The 2μm-family plasmid of ~~any preceding claim~~ Claim 1 additionally comprising a polynucleotide sequence insertion, deletion and/or substitution which is not at a position as defined in any one of the preceding claims.

19. (original) The 2μm-family plasmid of Claim 18 wherein the polynucleotide sequence insertion, deletion and/or substitution occurs within an untranscribed region around an ARS sequence.

20. (currently amended) The 2μm-family plasmid of ~~any one of the preceding claims~~ Claim 1 wherein the, or at least one, polynucleotide sequence insertion, deletion and/or substitution is a polynucleotide sequence insertion.

21. (original) The 2μm-family plasmid of Claim 20 in which the polynucleotide sequence insertion encodes an open reading frame.

22. (original) The 2μm-family plasmid of Claim 21 in which the open reading frame encodes a non-2μm-family plasmid protein.

23. (original) The 2μm-family plasmid of Claim 22 in which the non-2μm-family plasmid protein comprises the sequence of a protein involved in protein folding, or which has chaperone activity or is involved in the

unfolded protein response, albumin, a monoclonal antibody, an etoposide, a serum protein (such as a blood clotting factor), antistasin, a tick anticoagulant peptide, transferrin, lactoferrin, endostatin, angiostatin, collagens, immunoglobulins or immunoglobulin-based molecules or fragments of either (e.g. a dAb, Fab' fragments, F(ab')2, scAb, scFv or scFv fragment), a Kunitz domain protein, interferons, interleukins, IL10, IL11, IL2, interferon α species and sub-species, interferon β species and sub-species, interferon γ species and sub-species, leptin, CNTF, $CNTF_{Ax15}$, IL1-receptor antagonist, erythropoietin (EPO) and EPO mimics, thrombopoietin (TPO) and TPO mimics, prosaptide, cyanovirin-N, 5-helix, T20 peptide, T1249 peptide, HIV gp41, HIV gp120, urokinase, prourokinase, tPA, hirudin, platelet derived growth factor, parathyroid hormone, proinsulin, insulin, glucagon, glucagon-like peptides, insulin-like growth factor, calcitonin, growth hormone, transforming growth factor β, tumour necrosis factor, G-CSF, GM-CSF, M-CSF, FGF, coagulation factors in both pre and active forms, including but not limited to plasminogen, fibrinogen, thrombin, pre-thrombin, pro-thrombin, von Willebrand's factor, □$_1$-antitrypsin, plasminogen activators, Factor VII, Factor VIII, Factor IX, Factor X and Factor XIII, nerve growth factor, LACI, platelet-derived endothelial cell growth factor (PD-ECGF), glucose oxidase, serum cholinesterase, aprotinin, amyloid precursor protein, inter-alpha trypsin inhibitor, antithrombin III, apo-lipoprotein species, Protein C, Protein S, or a variant or fragment of any of the above.

24. (original) The 2μm-family plasmid of Claim 23 in which the non-

2μm-family plasmid protein comprises the sequence of albumin, a variant or fragment thereof, or a fusion protein comprising the sequence of any of these.

25. (original) The 2μm-family plasmid of Claim 23 in which the non-2μm-family plasmid protein comprises the sequence of transferrin, a variant or fragment thereof, or a fusion protein comprising the sequence of any of these.

26. (original) The 2μm-family plasmid of Claim 23 in which the non-2μm-family plasmid protein comprises the sequence of lactoferrin, a variant or fragment thereof, or a fusion protein comprising the sequence of any of these.

27. (original) The 2μm-family plasmid of Claim 23 in which the non-2μm-family plasmid protein comprises the sequence of Fc, a variant or fragment thereof, or a fusion protein comprising the sequence of any of these.

28. (original) The 2μm-family plasmid of Claim 23 in which the non-2μm-family plasmid protein comprises the sequence of a protein involved in protein folding, or which has chaperone activity or is involved in the unfolded protein response as encoded by any one of *AHA1*, *CCT2*, *CCT3*, *CCT4*, *CCT5*, *CCT6*, *CCT7*, *CCT8*, *CNS1*, *CPR3*, *CPR6*, *EPS1*, *ERO1*, *EUG1*, *FMO1*, *HCH1*, *HSP10*, *HSP12*, *HSP104*, *HSP26*, *HSP30*, *HSP42*, *HSP60*, *HSP78*, *HSP82*, *JEM1*, *MDJ1*, *MDJ2*,

MPD1, *MPD2*, *PDI1*, *PFD1*, *ABC1*, *APJ1*, *ATP11*, *ATP12*, *BTT1*, *CDC37*, *CPR7*, *HSC82*, *KAR2*, *LHS1*, *MGE1*, *MRS11*, *NOB1*, *ECM10*, *SSA1*, *SSA2*, *SSA3*, *SSA4*, *SSC1*, *SSE2*, *SIL1*, *SLS1*, *UBI4*, *ORM1*, *ORM2*, *PER1*, *PTC2*, *PSE1* and *HAC1* or a truncated intronless *HAC1*.

29. (currently amended) The 2μm-family plasmid of Claim 23 ~~or 28~~ in which the chaperone is protein disulphide isomerase (PDI), or is a protein encoded by *ORM2*, *SSA1* or *PSE1*.

30. (currently amended) The 2μm-family plasmid of ~~any one of~~ Claims 22 ~~to 29~~ in which the non-2μm-family plasmid protein comprises a secretion leader sequence.

31. (original) The 2μm-family plasmid of Claim 22 in which the non-2μm-family plasmid protein comprises the sequence of a bacterial selectable marker and/or a yeast selectable marker.

32. (original) The 2μm-family plasmid of Claim 31 in which the bacterial selectable marker is a β-lactamase gene and/or the yeast selectable marker is a *LEU2* selectable marker.

33. (currently amended) The 2μm-family plasmid according to ~~any one of the preceding claims~~ Claim 1 which plasmid comprises (i) a heterologous sequence encoding a non- 2μm-family plasmid protein; (ii) a heterologous sequence encoding a protein comprising the sequence of a protein involved in protein folding, a chaperone or a

protein involved in the unfolded protein response, preferably protein disulphide isomerase; and (iii) a heterologous sequence encoding a protein comprising the sequence of a selectable marker; wherein at least one of the heterologous sequences occurs at a position as defined by any one of Claims 1 to 16.

34. (currently amended) A method of preparing a plasmid as defined by ~~any one of the preceding claims~~ Claim 1 comprising

(a) providing a plasmid comprising the sequence of a *REP2* gene and the inverted repeat that follows the *REP2* gene, or a *FLP* gene and the inverted repeat that follows the *FLP* gene, in each case the inverted repeat comprising an FRT site;

(b) providing a polynucleotide sequence and inserting the polynucleotide sequence into the plasmid at a position as defined in ~~any one of~~ Claims 1 ~~to 16~~; and/or

(c) deleting some or all of the nucleotide bases at the positions defined in ~~any one of~~ Claims 1 ~~to 16~~; and/or

(d) substituting some or all of the nucleotide bases at the positions defined in ~~any one of~~ Claims 1 ~~to 16~~ with alternative nucleotide bases.

35. (original) A plasmid obtainable by the method of Claim 34.

36. (currently amended) A host cell comprising a plasmid as defined by ~~any one of~~ Claims 1 ~~to 33 and 35~~.

37. (original) A host cell according to Claim 36 which is a yeast cell.

38. (currently amended) A host cell according to Claim 36 ~~or 37~~ in which the plasmid is stable as a multicopy plasmid.

39. (original) A host cell according to Claim 38 in which the plasmid is based on pSR1, pSB3 or pSB4 and the yeast cell is *Zygosaccharomyces rouxii*, the plasmid is based on pSB1 or pSB2 and the yeast cell is *Zygosaccharomyces bailli*, the plasmid is based on pSM1 and the yeast cell is *Zygosaccharomyces fermentati*, the plasmid is based on pKD1 and the yeast cell is *Kluyveromyces drosophilarum*, the plasmid is based on pPM1 and the yeast cell is *Pichia membranaefaciens* or the plasmid is based on the 2μm plasmid and the yeast cell is *Saccharomyces cerevisiae* or *Saccharomyces carlsbergensis*.

40. (currently amended) A host cell according to Claim 38 ~~or 39~~ in which, if the plasmid contains, or is modified to contain, a selectable marker then stability, as measured by the loss of the marker, is at least 1%, 2%, 3%, 4%, 5%, 10%, 15%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.9% or substantially 100% after 5 generations.

41. (currently amended) A method of producing a protein comprising the steps of –

(a) providing a plasmid as defined by ~~any one of~~ Claims 1 ~~to 33 or 35~~;

(b) providing a suitable host cell;

(c) transforming the host cell with the plasmid; and

(d) culturing the transformed host cell in a culture medium;

(e) thereby to produce the protein.

42. (currently amended) A method of producing a protein comprising the steps of providing a host cell as defined by ~~any one of~~ Claims 36 ~~to 40~~ which host cell comprises a plasmid as defined by ~~any one of~~ Claims 1 ~~to 33 or 35~~ and culturing the host cell in a culture medium thereby to produce the protein.

43. (currently amended) A method according to Claim 41 ~~or 42~~ further comprising the step of isolating the thus produced protein from the cultured host cell or the culture medium.

44. (original) A method according to Claim 43 further comprising the step

of purifying the thus isolated protein to a commercially acceptable level of purity.

45. (original) A method according to Claim 44 further comprising the step of formulating the thus purified protein with a carrier or diluent, and optionally presenting the thus formulated protein in a unit form.

46. (original) A method according to Claim 43 further comprising the step of purifying the thus isolated protein to a pharmaceutically acceptable level of purity.

47. (original) A method according to Claim 44 further comprising the step of formulating the thus purified protein with a pharmaceutically acceptable carrier or diluent and optionally presenting the thus formulated protein in a unit dosage form.

48 – 63. (canceled).